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PATENT- OG VAREMÆRKESTYRELSEN

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HAEMOSTATIC SPONGE COMPRISING HYALURONIC ACID

FIELD OF THE INVENTION

The present invention relates to a haemostatic sponge comprising a biologically absorbable solid material, preferably gelatine, and hyaluronic acid or a derivative thereof. Such haemostatic sponges swell to a much lesser extent than conventional sponges based solely on, e.g., gelatine. In addition, the haemostatic sponges described herein have superior haemostatic properties as compared to conventional sponges.

BACKGROUND OF THE INVENTION

When blood vessels are injured by physical traumas including surgical interventions bleedings will occur. Dependent on the extent of the injury, bleedings may result in losses of blood which can affect the normal function of the individual or, in cases of bleedings occurring in osseous non-expandable cavities, the accumulation of extravasated blood may cause damages of soft tissues due to increased pressure. If bleedings are left alone they will eventually be arrested by a normally occurring physiological process characterised by a chain of events involving the combined activity of vascular, platelet, and plasma factors. This process is referred to as a physiological haemostasis, an important element of which is blood coagulation which is described below. In the case of a minor superficial bleeding this physiological haemostasis is adequate for the arrest.

Blood coagulation may be described as occurring in the following steps.

(1) The formation of an activator of prothrombin, which is a precursor of the plasma serine protease thrombin. The prothrombin activator is a complex of an enzyme factor Xa and two cofactors: factor Va and procoagulant phospholipids, both present on the surface of activated platelets. Furthermore, the presence of calcium ions is necessary for the function of the activator.

(2) The cleavage by the above activator system of prothrombin into two fragments, one of which is the enzyme thrombin.

(3) The conversion by thrombin of the plasma precursor fibrinogen to the clotting substance fibrin. This process involves several steps, the first of which comprises the cleavage of small peptides from fibrinogen, whereby fibrin monomers are formed, which then polymerise to form insoluble fibrin polymers. As a final step, thrombin activates the plasma factor XIII, an enzyme that catalyses the formation of covalent bonds between fibrin molecules, thereby cross-linking the molecules to form a firm clot resistant to dissolution.

In the above step (1) leading to the formation of the prothrombin activator system, several plasma proteases are involved in a cascade of proteolytic events. These blood coagulation factors are currently referred to by using Roman numerals, such as factor VII, factor VIII, factor IX, factor XI, and factor XII. The cascade involves sequential proteolytic activations of the next enzyme in the cascade. Thus activated blood coagulation factors are designated by their Roman numerals followed by an "a", such as factor VIIa, factor VIIIa, factor XIIIa or factor IXa.

However, bleedings emerging from more extensive injuries, especially such injuries which involve larger arteries or when seeping bleedings occur from larger mucosal surfaces or in cavities without drainage, require the adoption of surgical and/or medical haemostatic measures. Surgical arrest of bleeding comprise ligation or suture of disrupted blood vessels, plugging by using tampons in cavities, coagulating tissue surfaces including their exposed disrupted blood vessels by heated instruments or by the application of cauterising agents or heated air. Surgical haemostasis may also be aided by the application at the injured site of appropriately sized blocks, plates, or films of biologically absorbable haemostatic sponges.

In this context, the term "sponge" is understood to mean a porous structure characterised in that the structure is reticulate and has an inner surface considerably larger than its outer surface, that it contains hollow spaces within the reticulate structure, and that it can absorb many times its own weight in liquids.

Such haemostatic sponges are useful for enhancing the arrest of bleedings in several instances of surgical interventions or other injuries such as in surgery of large abdominal organs (liver, spleen, or intestines); in lung surgery; in neurosurgery to prevent pressure damages of the cerebral or nerve tissues; in orthopedic surgery during which extensive haemorrhages frequently occur which are difficult to arrest by other means; in vascular surgery to arrest seeping bleedings from the sites of suturing; in oral or dental surgery such as extraction of teeth; and in nose-bleeding (epistaxis).

It is currently believed that the haemostatic effect (or mode of action) of a sponge is linked to sponge porosity and the sponge's ability to absorb blood. A conventional gelatine sponge adheres to the bleeding site and absorbs approximately 45 times its own weight. Due to the uniform porosity of a conventional gelatine sponge, blood platelets are caught and the coagulation cascade is activated transforming soluble fibrinogen into a net of insoluble fibrin which stops the bleeding. Thus, a good capacity to absorb is believed essential for the mode of action of conventional gelatine sponges.

As mentioned above, a conventional gelatine sponge's ability to act as a haemostat is related to its ability to absorb, whereby the volume of the sponge inevitably will increase. However, swelling of the sponge can lead to adverse events if the sponge is not used according to the instructions. Normally, the instruction for use includes the phrase: "When placed into cavities or closed tissue spaces, minimal preliminary compression is advised and care should be exercised to avoid overpackaging. The gelatine sponge may swell to its original size on absorbing fluids creating the potential for nerve damage". Nevertheless, adverse events have taken place in the past, and the UK Medical Device Agency as well as the FDA have paid much attention to this draw back of conventional sponges.

Accordingly, there is a need for haemostatic sponges which, while maintaining a sufficient blood arresting (haemostatic) effect, swell to a much lesser extent than conventional haemostatic sponges. Evidently, such sponges would constitute a safer product.

The present inventors have solved the above-mentioned problem by incorporating hyaluronic acid (HA), or a derivative thereof, into or onto the haemostatic sponge.

Surprisingly, it has been found that swelling of such haemostatic sponges is considerably reduced as compared to conventional sponges, such as Surgifoam®. Evidently, the haemostatic sponges described herein are safer to use than conventional sponges.

In addition, it has surprisingly been found that the haemostatic sponges of the present invention are more efficient in arresting bleeding as are conventional sponges, such as Surgifoam®, i.e. the haemostatic properties of the sponges according to the invention are improved compared to conventional sponges.

The above-mentioned properties, i.e. decreased tendency to swell and improved haemostatic properties, are attributed to the presence of HA, or derivatives thereof. HA, and derivatives thereof, are known to confer anti-adhesive properties to sponges as described in e.g. Laurent et al. *Am J Otolaryngol;* 7:181-186, 1986; US 6,548,081; US 6,099,952; US 5,503,848; US 5,700,476; EP 1 022 031 A1; WO 94/17840. Nevertheless, the surprising effects of conferring improved haemostatic properties to the sponge and reducing the ability of the sponge to swell, have not been described in any of the above-identified prior art documents.

In summary, the haemostatic sponges of the present invention contain numerous advantages as compared to conventional and commercially available haemostatic sponges:

i) reduced swelling, thereby rendering the sponge safer to use,

ii) improved haemostatic properties, and

iii) improved anti-adhesive properties, thereby reducing post-operative adhesion of tissues.

SUMMARY OF THE INVENTION

In a first aspect, the present invention relates to a haemostatic sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof.

Other aspects of the present invention are directed to methods for producing the haemostatic sponges of the invention as well as to their medical uses. These, and other, aspects will be apparent from the below disclosure and the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

The general effect of haemostatic sponges is to enhance the physiological blood coagulation process thereby reducing the time elapsing from opening of the blood vessels until a firm blood clot has been formed. This period is generally referred to as "the blood coagulation time". In this context, the term "haemostatic" should be understood to mean the effect of an object or an agent which reduces the blood coagulation time, thereby promoting haemostasis.

As indicated above, the present invention is, in its broadest aspect, directed to a haemostatic sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof.

The biologically absorbable material may be any material, which is known to be suitable for preparation of sponges and, at the same time, being biologically absorbable. Examples of suitable biologically absorbable materials include gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof. It will be understood that various forms thereof, such as linear or cross-linked forms, salts, esters and the like may also be used as the biologically absorbable material to be included in the haemostatic sponges of the invention.

"Biologically absorbable" is a term which in the present context is used to describe that the materials of which the said sponges are made can be degraded in the body to smaller molecules having a size which allows them to be transported into the blood stream. By said degradation and absorption the said sponge materials will gradually be removed from

the site of application. For example, denatured gelatine can be degraded by proteolytic tissue enzymes to absorbable smaller molecules, whereby the denatured gelatine sponge when applied in tissues typically is absorbed within about 3-6 weeks and when applied on bleeding surfaces and mucous membranes typically within 3-5 days.

In a preferred embodiment of the invention, the biologically absorbable material is gelatine. Gelatine is preferred since gelatine is highly biologically absorbable. Furthermore, gelatine is highly biocompatible, meaning that it is non-toxic to an animal, such as a human being, when/if entering the blood stream or being in long-term contact with human tissues.

The gelatine typically originates from a porcine source, but may originate from other animal sources, such as from bovine or fish sources. The gelatine may also be synthetically made, i.e. made by recombinant means. In an interesting embodiment, the gelatine is denatured, e.g. by heating gelatine in air at a temperature in the range of 100°C to 160°C, preferably at a temperature of about 150°C for about 0.5 to 4 hours. Alternatively, the gelatine may be denatured by chemical treatment with acids, bases, solvents, aldehydes, urea, or detergents such as sodium dodecyl sulphate and guanidine hydrochloride. By the above-mentioned denaturation procedure, the chemical characteristics of the gelatine molecule are modified, thereby resulting in loss of water solubility.

Furthermore, the gelatine may be hardened, which is considered advantageous in relation to the use of the sponge as a haemostat, the mechanical strength of the structure being greatly increased as compared to a non-denatured gelatine structure which when becoming moistened will become dissolved and thereby collapse. In contrast, a hardened, denatured gelatine sponge will retain its structure for a considerable period of time after application to a bleeding site.

Even though denatured gelatine as defined above represents a particularly suitable embodiment of the present invention, it will be understood that other biodegradable materials currently used for haemostatic purposes, such as collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof, said materials being in their native form or structurally modified, may also be used without being regarded as departures from the spirit and scope of the present invention.

As indicated previously, the advantageous properties of the haemostatic sponges of the present invention are attributed to the presence of HA, or derivatives thereof, in the sponge. Another inherent advantage of HA, or a derivative thereof, is the excellent biologically absorbable and biocompatible properties of the molecule.

HA is a natural heteropolysaccharide consisting of alternate residues of D-glucuronic acid and N-acetyl-D-glucosamine. It is a linear polymer having a molecular weight ranging from about 50 to about 13,000 kDa, depending on the source it is obtained from and on the method of preparation. HA is present in nature in the preicellular gels, in the fundamental substance of connective tissue in vertebrate organisms (of which it is one of the main components), in the synovial fluid of joints, in the vitreous humor and in the umbilical cord. HA plays an important role in the biological organism as mechanical support for the cells of many tissues such as the skin, the tendons, the muscles and the cartilage. It is the main component of the extracellular matrix, but it has other functions such as hydration of tissues, lubrication as well as cell migration and differentiation. A suitable molecular weight for the purposes described herein will be in the range of from 50 to 5,000 kDa, such as in the range of from 50 to 4,000 kDa, e.g. in the range of from 100 to 3,000 kDa. In a particular preferred embodiment of the invention, the HA, or a derivative thereof, has a molecular weight in the range of from 250 to 2,500 kDa, more preferably in the range of from 500 to 2,000 kDa.

Optionally, the HA molecule may be cross-linked, e.g. by chemical or physical means. In a preferred embodiment of the invention the employed HA is pH neutral, i.e. an aqueous solution of the employed HA exhibits a pH value in the range of from 5 to 9, preferably in the range of from 6-8, in particular in the range of from 6.5 to 7.5, such as about 7. The HA used in the present invention may be extracted from any source, for example from cocks' comb. Alternatively the HA may be obtained by fermentation.

Derivatives of HA include, for example, esters of HA, as well as the derivatives described in US 5,356,883; US 6,548,081; US 4,851,521; US 6,027,741; US 2003 181689; EP 1 095 064; EP 0 341 745; V/O 02/18450 and WO 2004/035629. In addition, the term "derivative" is also intended to cover hyaluronate salt, including, but not limited to, sodium hyaluronate, potassium hyaluronate, magnesium hyaluronate and calcium hyaluronate.

Specific examples of HA derivatives include the following HA derivatives:

HA salified with organic and/or inorganic bases,

Hyaff®, i.e. HA esters with alcohols of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series, with an esterification degree that may vary depending on the type and length of the alcohol used,

Hyadd®, i.e. amides of HA with amines of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series, with an amidation degree that may vary depending on the type and length of the amine used,

Hyoxx®, i.e. percarboxylated HA derivatives obtained by oxidation of the primary hydroxyl group of the N-acetyl-D-glucosamine unit,

deacetylates of HA, i.e. derived from deacetylation of the N-acetyl-D-glucosamine unit, and

O-sulphated HA derivatives.

It is a requirement that the haemostatic sponge of the present invention contains at least 10% (w/w), calculated on the basis of the total weight of the dry sponge, of HA or a derivative thereof. In an interesting embodiment of the invention, the haemostatic sponge of the invention comprises at least 15% (w/w) of HA or a derivative thereof, such as at least 20% (w/w) of HA or a derivative thereof, e.g. at least 25% (w/w) of HA or a derivative thereof, preferably at least 30% (w/w) of HA or a derivative thereof, such as at least 35% (w/w) of HA or a derivative thereof, e.g. at least 40% (w/w) of HA or a derivative thereof.

Analogously, the haemostatic sponge of the invention typically comprises at the most 85% (w/w) of said biologically absorbable solid material, such as at the most 80% (w/w) of said biologically absorbable solid material, e.g. at the most 75% (w/w) of said biologically absorbable solid material, preferably at the most 70% (w/w) of said biologically absorbable solid material, such as at the most 65% (w/w) of said biologically absorbable solid material, e.g. at the most 60% (w/w) of said biologically absorbable solid material.

As will be understood by the skilled person, a significant amount of the biologically absorbable solid material must, however, be present in the sponge in order to provide the sponge with satisfactory mechanical and structural properties, i.e. the amount of HA, or a derivative thereof, should preferably not be too high. Accordingly, in a preferred embodiment of the invention, the sponge comprises at the most 90% (w/w) of HA or a derivative thereof, such as at the most 80% of HA or a derivative thereof, e.g. at the most 70% (w/w) of HA or a derivative thereof, preferably at the most 65% (w/w) of HA or a derivative thereof, such as at the most 60% (w/w) of HA or a derivative thereof, e.g. at the most 55% (w/w) of HA or a derivative thereof, in particular at the most 50% (w/w) of HA or a derivative thereof.

Some residual water, such as up to about 10% (w/w) may be present in the sponge. This residual water is, however, not calculated as part of the total weight of the sponge. This means that when the sponge is said to contain a specified weight percent of a certain component (such as the HA, a derivative thereof or the biologically absorbable solid material) this weight percent is calculated on the basis of the total dry weight of the sponge.

In the currently most preferred embodiment of the invention, the HA, or a derivative thereof, is incorporated in the haemostatic sponge. By the term "incorporated" is to be understood that the HA molecules are located more or less uniformly in the reticulate sponge structure in such a way that no "hot spots" of the HA molecules can be found in the sponge. Thus, the term "incorporated", when used herein, may be used synonymously with expressions like "absorbed", "admixed" and the like.

In another embodiment of the invention, the HA, or a derivative thereof, is applied to one or more of the surfaces of the sponge. In a preferred embodiment, the HA, or derivative thereof, is not applied to the surface intended for being in direct contact with the bleeding site, i.e. in a preferred embodiment the HA, or a derivative thereof, is applied to only one, two or three of the surfaces of the sponge. It will be understood that when the HA, or a derivative thereof, is applied to one or more of the surfaces of the sponges, the major part of the HA, or a derivative thereof, will be located on said surface(s). Nevertheless, a certain amount of HA, or a derivative thereof, may end up being incorporated into the sponge as the pore size of the sponge may be so that when a layer of HA, or a derivative thereof, is applied to the surface(s) of the sponge, the molecules of the HA, or a derivative thereof, may partially penetrate into the pores of the sponge.

Even though HA, or derivatives thereof, represents a particularly suitable embodiment of the present invention, it will be understood that other polysaccharides with properties similar to HA may be used in the present invention without being regarded as departures from the spirit and scope of the present invention. Examples of polysaccharides which may substitute HA, or derivatives thereof, in the sponges of the invention, include the mucopolysaccharides, such as chitin, chitosan, chondroitin sulphate, dermatan sulphate, keratan sulphate as well as alginate.

As explained above, one particular advantage of the haemostatic sponge of the invention is the anti-adhesive properties of HA, or a derivative thereof, which in turn has the advantage that post-operative adhesion of tissues may be avoided as explained in, e.g. US 5,548,081 and in Laurent et al. *Am J Otolaryngol;* 7:181-186, 1986.

The main advantage of the haemostatic sponges of the present invention is, however, their ability to provide an efficient haemostatic action while, at the same time, possessing a reduced tendency to swell. Swelling of haemostatic sponges may be measured by various methods. For example, the swelling tendency may be expressed as the sponge's capability to absorb water; the more water absorbed, the more swelling (the greater volume) is obtained.

As will be apparent from the experiments provided herein, the haemostatic sponge of the invention has a reduced tendency to swell, i.e. to absorb water, when determined in accordance with USP 24. Accordingly, a preferred haemostatic sponge of the invention absorbs less water, i.e. it swells to a lesser extent, than an absorbable gelatine sponge, such as Surgifoam®. More particularly, in a preferred embodiment of the invention, the ratio between the water absorbed by the haemostatic sponge of the invention and the water absorbed by an absorbable gelatine sponge, such as Surgifoam®, is at the most 0.80 when determined in accordance with USP 24. More preferably, the ratio is at the most 0.75, such as at the most 0.70, e.g. at the most 0.65, even more preferably at the most 0.60, such as at the most 0.55, in particular at the most 0.50.

Alternatively, the swelling properties may be assessed by soaking the haemostatic sponge in an excessive amount of distilled water. The sponge is subsequently picked up and air-dried for 10 minutes in order to drain out excess free water. The weight of the sponge before and after water absorption is used for calculating the swelling ratio: Swelling ratio = $(m_f - m_i)/m_i$, where m_f is the weight of the sponge after soaking and removing of excess water, and m_i is the initial weight of sponge before soaking.

Alternatively or in addition to the above tests, one may measure the expanding volume of a sponge in a graduated cylinder with distilled water; A known weight and volume of a sponge is pre-wetted and kneaded and quantitatively poured into the cylinder. The expanded volume is then measured and divided by the initial mass of the sponge, i.e. the weight of the sponge before pre-wetting.

In a preferred embodiment of the invention, the sponge further comprises at least one blood coagulation factor, such as a blood coagulation factor selected from the group consisting of thrombin or a precursor therefor, factor Va, factor VIIa, factor VIIIa, factor IXa, factor Xa, factor XIa, factor XIIa, factor XIIIa and calcium ions. In a highly preferred embodiment of the invention, said blood coagulation factor is thrombin or a precursor therefor, in particular thrombin itself. The thrombin may be plasma-derived, typically from a mammal source, such as a human. Alternatively, the thrombin may be produced by recombinant means.

Thrombin as well as precursors therefor may be incorporated into a haemostatic sponge by conventional means known to the person skilled in the art and as carefully explained in WO 90/13320.

In case thrombin or a precursor therefor is incorporated into the sponge of the invention, the sponge preferably further comprises a thrombin-stabilising agent, such as a thrombin-stabilising agent selected from the group consisting of naturally occurring amino acids, mono- or disaccharides, polyglycols, proteins and mixtures thereof.

By naturally occurring amino acids is understood any amino acid which is found in biologically produced proteins, including essential and non-essential dietary amino acids in their two stereoisomeric forms, such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, aspartate, cysteine, glutamate, glycine, proline, serine, tyrosine, glutamine, and asparagine. Preferred amino acids are selected from the group consisting of glycine, lysine and arginine.

Suitable monosaccharides may be selected from D- or L-forms of pentoses, such as ribose, arabinose, xylose, and lyxose and hexoses such as allose, altrose, glucose, mannose, gulose, idose, galactose, talose and derivatives thereof, e.g. pentosamines, hexosamines and glucoronic acid. Disaccharides may be selected from lactose, saccharose, maltose, fructose, and cellubiose, including derivatives thereof.

In a preferred embodiment of the sponge of the invention, a polyvalent alcohol is used as a thrombin-stabilising agent. A suitable polyvalent alcohol may be selected from ethylene glycol, diethylene glycol, propylene glycol, glycerol, mannitol, inositol, xylitol, erythritol, pentaerythritol, pentitols, hexitols, such as sorbitol, and heptitols. Furthermore, polyglycols, such as polypropylene glycol and polyethylene glycols may be useful as thrombin-stabilising agents. Among the latter group of compounds polyethylene glycols having a molecular weight in the range of 400 - 20,000, such as about 6,000 are preferred.

In an interesting embodiment of the invention, the HA, or derivative thereof, exerts in itself a thrombin-stabilising effect, in which case the haemostatic sponge does not contain any further thrombin-stabilising agents.

The mammalian body has an innate fibrinolytic system which is activated by deposition of fibrin. By dissolving fibrin, this system helps keep open the lumen of an injured blood vessel. However, in a situation where rapid haemostasis is aimed at, the fibrinolytic activity may counteract the haemostatic effect of a haemostatic adjunct, such as a

haemostatic sponge according to the present invention. The fibrinolytic system involves the activation of plasminogen, a plasma precursor for an active proteolytic enzyme, plasmin, which is bound to lysine residues on the fibrin. Accordingly, it may be advantageous to have agents incorporated in the haemostatic sponge of this invention, which have an anti-fibrinolytic effect. Specific examples include an anti-fibrinolytic agent selected from the group consisting of aprotinin, pepstatin, leupeptin, antipain, chymostatin, gabexate mesilate, fibronectin, ε-amino caproic acid and tranexamic acid. Most preferably, the anti-fibrinolytic agent is ε-amino caproic acid or tranexamic acid, in particular tranexamic acid.

In addition, the haemostatic sponge of the invention may contain a buffering agent. Examples of buffering agents include alkaline metal salts, such as acetates, citrates, phosphates, hydrogen phosphates, carbonates, hydrogen carbonates, and succinates. Other useful buffering agents include imidazole, TRIS, and zwitteranionic buffering systems. Evidently, mixtures of the above-mentioned buffering agents may also be used.

Due to the superior swelling properties of the sponges describe herein, it will be appreciated that the sponge in addition to, or as an alternative to, providing a haemostatic effect, also may be used for local delivery of desirable agents, thereby using the sponge as a delivery vehicle or matrix. The desirable agent may be incorporated in the sponge or applied to one or more of the surfaces of the sponge in a conventional way, e.g. by soaking, dipping, spraying the sponge of the invention in or with a solution of the desirable agent or by other methods known to the skilled person. This can, e.g., be done by the clinician prior to use of the product by the clinician, or by the manufacturer prior to finishing the sponge.

Accordingly, in another aspect the present invention relates to sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, in particular a desirable agent selected from the group consisting of surfactants, antimicrobial agents, antibacterial agents such as antiseptics and antibiotics, pain relieving agents, chemotherapeutics, anaesthetics, healing-promoting agents, vitamins, minerals, amino acids, proteins, growth factors, cells, enzymes, contrast agents, preservatives, emulsifiers, cross-linking agents to promote healing, etc.

In yet another aspect, the present invention relates to the use of a sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, for local delivery of said desirable agent.

In still another aspect, the present invention relates to a method for local delivery of a desirable agent to a patient in need thereof, said method comprising placing a sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, at the local site of the patient where said desirable agent is intended to be delivered.

The term "local site" is intended to mean a part of a patient's body, in particular internal organs, such as a kidney, spleen, heart, etc.

Antimicrobial agents may be selected from bactericidal or bacteriostatic agents, such as antibiotics and sulphonamides, antiviral compounds, antimycotic agents and anti-infectives. Antibiotics may be selected from e.g. β-lactams, penicillins, cephalosporins, monobactams, macrolides, polymyxins, tetracyclines, chloramphenicol, thrimethoprim, aminoglycosides, clindamycin, and metronidazole; sulphonamides may as an example be selected from sulphadimidine or sulphadimethoxin; antimycotic agents may be selected from amphotericin B, ketoconazol and miconazol; and antiviral agent from idoxuridine andazidothymidin. Suitable antiinfectives may as an example be selected from halogens, chlorohexidine, quarternary ammonium compounds and triclosan. Other examples of bactericidal or bacteriostatic compounds include silver ions, in particular in the form of silver ion complexes.

Surfactants may be selected from the group consisting of anionic surfactants, cationic surfactants, non-ionic surfactants and surface active biological modifiers.

Examples of anionic surfactants include surfactants selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose. In particular sodium lauryl sulfate is preferred.

Examples of cationic surfactants include surfactants selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride.

Examples of non-ionic surfactants include surfactants selected from the group consisting of polyoxyethylene fatty a cohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone.

Examples of biological surfactants include, e.g., albumin and casein.

Examples of preservatives include benzoic acid, sorbic acid, parabens (e.g. methyl-p-hydroxy benzoic acid, ethyl-p-hydroxy benzoic acid, propyl-p-hydroxy benzoic acid, butyl-p-hydroxy benzoic acid and mixtures thereof), benzyl alcohol, chlorhexidine or benzalkonium chloride.

In an interesting embodiment of the invention, the haemostatic sponge of the invention is equipped with a top sheet, i.e. at least one of the surfaces of said haemostatic sponge may be covered by a top sheet. In one embodiment of the invention, the top sheet is removable and constructed from a thin plastic film of e.g. polyethylene, polypropylene or other materials which are substantially water-impervious. The skilled person will be aware of other suitable materials having the desired and required mechanical properties for this purpose. As will be understood, such top sheets are typically not biodegradable and should subsequently be removed. The materials mentioned above are typically transparent which, in turn, may give rise to problems in identifying the top sheet under and/or after surgery, in particular if the surgical area is covered or filled with a substantial amount of blood. Evidently, this increases the risk that the surgeon, or the staff assisting him, overlooks the presence of the top sheet. As will be understood, in case such a non-biodegradable top sheet is left in the body this may give rise to a severe clinical condition for the patient in question. Accordingly, in a preferred embodiment of the invention, a dye is incorporated in or on the top sheet, or part of the top sheet, thereby improving the visibility of the top sheet.

In another, and even more preferred, embodiment of the invention, the top sheet is prepared from a biodegradable material. Examples of suitable biodegradable materials include, for example, the polymeric materials mentioned in WO 2004/028583, page 6, line 3 to page 7, line 32, which is incorporated herein by reference. It will be understood that in this case the top sheet needs not necessarily be removed after surgery but may be left in the body.

The sponges of the invention may be prepared in any desirable shape and/or dimension depending on the intended use and the available process equipment. Typically, however, the thickness of the sponge of the invention will be in the range of 1-20 mm, such as in the range of 2-10 mm. Preferably, the thickness of the sponge is not less than 1 mm.

The haemostatic sponge is preferably subjected to a sterilisation treatment by application of radiation, such as β-radiation. The dose typically lies in the range of 20-60 kGy, e.g. 25 kGy. Such treatment will reduce the bioburden of the sponge, and may also add to cross-linking of the molecular chains in the product. This may, however, be counteracted by break-down of bonds in the linear chain molecules.

As indicated above, the haemostatic sponge of the invention may be used as a medicament. In particular, the haemostatic sponge of the invention may be used for a haemostatic adjunct, or in the preparation for a haemostatic adjunct, in medical, veterinary or dental surgery. Accordingly, in a further aspect the present invention relates to a method of promoting haemostasis in a patient in need thereof, said method comprising applying a haemostatic sponge of the invention onto at least a portion of the area where bleeding occurs. In a still further aspect the present invention relates to a method for arresting bleeding comprising applying to the site of bleeding a haemostatic sponge according to the invention.

The haemostatic sponge may be applied directly to surfaces and optionally, after being applied to the surface, held in place by pressure, e.g. by means of pads, dressings, webs, films, etc. or by other materials normally used in the medical practice. A preferred material for holding the sponge in place after being applied to the surface is surgical gauze or cotton gauze, optionally wetted in saline.

The haemostatic sponge of the invention may be used in an array of surgical procedures wherein bleeding control is desired, such as in orthopedic precedures, e.g. in connection with laminectomy, total hip replacement and hip revisions, knee surgery, spinal fusion, etc.; in cardiothoracic/cardiovascular procedures, such as in connection with CABGs, valve replacements, aotic surgery, abdominal aortic aneurisms, carotid endarterectomy and femoral-popliteal bypass, amongst others.

In a further aspect the original shape of the haemostatic sponges of the invention may be modified. For example, the sponge of the invention may be milled to a powder or flakes by methods known in the art, e.g. by means of rotary bed, extrusion, granulation and treatment in an intensive mixer, milling (e.g. by using a hammer mill or a centrifugal mill),

or spray drying. Such powders or flakes may be used "as is" or may be pre-wetted with a liquid, such as saline, before use, thereby creating a paste.

The term "paste" may be used interchangeable with words like "gel", "suspension" and the like. In the present context, the term "paste" refers to a solid or semi-solid disperse system wherein the biologically absorbable solid material is dispersed in a liquid medium. The biologically absorbable solid material may also be referred to as a gel- or paste-forming agent. Furthermore, a paste is characterised by having a dynamic viscosity above that of water.

The paste may be obtained by suspending the particles of the biologically absorbable solid material (described above) in a liquid medium, in particular in an aqueous medium. Typically, about 1-20 ml liquid medium is employed per gram biologically absorbable solid material. The liquid medium is preferably an aqueous medium. More preferably the aqueous medium contains salts, such as sodium chloride, dissolved therein. Most preferably, the aqueous medium is saline.

Accordingly, in a further aspect the present invention relates to a powder composition comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof.

In a still further aspect the present invention relates to a paste comprising water, a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof. It will be understood the weight percentages given is calculated on the basis of only solid material in the paste.

Details and particulars concerning the powder and paste aspects will be the same as for the sponge aspect discussed above, and this means that, whenever appropriate, the statements concerning amounts of HA, or a derivative thereof, present in the sponge, additional components present in the sponge, etc., will apply mutatis mutandis to the powder and paste aspects of the invention.

In further aspects the present invention relates to methods for preparing the haemostatic sponges of the invention.

More particularly, the present invention relates to a method for preparing a haemostatic sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is incorporated in said sponge, said method comprising the steps of:

i) providing a whipped or foamed solution of a biologically absorbable solid material selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof;

ii) providing a solution of HA or a derivative thereof;

iii) mixing the solutions provided in i) and ii) above; and

iv) drying said mixture.

Concerning step i) above, the preferred biologically absorbable material is gelatine. It is important that the solution is foamed, whipped or subjected to other mechanical forces, so that the desirable texture is obtained. In a preferred embodiment of the invention, the texture of the whipped or foamed solution resembles that of whipped cream. A suitable concentration of the biologically absorbable material will depend on the material of choice, but will typically be in the range of from 5 to 30% (w/v), such as in the range of from 10 to 20% (w/v). The temperature is preferably kept in the range of from 25°C to 60°C, more preferably in the range of from 35°C to 55°C.

The HA solution mentioned in step ii) above is preferably provided in the form of a gel. The exact concentration of HA, or a derivative thereof, may largely depend on the HA or derivative used. Generally speaking, the solution should neither be too liquid, nor too viscous. The present inventors have found that good results are obtained using a concentration of from 1 to 5% (w/v), in particular of from 2 to 4% (w/v). In order to avoid cooling of the whipped or foamed solution of biologically absorbable material upon mixing, thereby potentially leading to an inhomogenous mixture, the HA solution is preferably kept at an slightly elevated temperature, such as a temperature in the range of from 25°C to 50°C, e.g. in the range of from 25°C to 35°C.

The mixing step iii) above is typically performed under vigorous mechanical influence in order to avoid clogging of the biologically absorbable material. Thus, during and/or immediately after mixing, the resulting mixture should preferably be whirled at high velocity, whipped, spinned, centrifuged or subjected to other kind of mechanical influence as will be known to the person skilled in the art.

After mixing the resulting mixture may be poured into suitable trays or placed on finely perforated teflon sheets and drying is then performed at a temperature of from about 25°C to about 35°C, such as about 30°C, for about 12 to about 24 hours, typically for about 16

hours. If desired, the resulting sponge material may be hardened by dry heat at elevated temperatures, such as in the range of from about 110°C to about 160°C. The hardening time depends on the temperature, but will typically be from about 30 minutes to several hours.

In an alternative embodiment, the sponge is prepared by freeze-drying a mixture of the two solutions.

In still another aspect the present invention relates to a method for preparing a haemostatic sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is applied to one or more of the surfaces of the sponge, said method comprising the steps of:

i) providing a sponge comprising a biologically absorbable solid material selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof;

ii) providing a solution of HA or a derivative thereof;

iii) applying said solution of HA, or a derivative thereof, to one or more of the surfaces of the sponge; and

iv) drying the resulting sponge.

In a similar way as described above, the sponge is preferably a gelatine sponge, such as the commercially available Surgifoam® sponge. The HA solution as well as the drying step is preferably as discussed above. The HA solution may be applied to one or more of the surfaces of the sponge by any conventional technique known the person skilled in the art.

The present invention is further illustrated by the following, non-limiting, examples.

EXPERIMENTAL

Determination of water absorption according to USP 24

Cut a portion of about 10 mg from a absorbable gelatine sponge, weigh accurately, and place in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all air has been removed, taking care not to break the tissue. Lift the portion of sponge from the water, and blot twice by pressing firmly between two pieces of absorbent paper. Drop the expressed sponge into a tared weighing bottle containing about 20 ml of water, and allow standing for 2 minutes. Lift the sponge from the water with a suitable hooked instrument, allow draining for 5 seconds, and discard the sponge. Again weigh the weighing bottle and water: the loss in water represents the weight of water absorbed by the sponge.

Example 1 - Preparation of sponge #1

A HA gel (2-4% (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was added to freshly foamed gelatine (16.7% (w/v)). Immediately after addition of HA, the mixture was whirled at high velocity to avoid clogging of the gelatine. In order to avoid an inhomogeneous mixture, the temperature should not be below room temperature. After mixing, the mixture was poured into trays or placed on finely perforated teflon sheets, followed by heat drying at approximately 30°C and 10% relative humidity for about 16 hours. Sponges prepared this way typically had a HA content of about 25-50% (w/w).

Example 2 - Preparation of sponge #2

A HA gel (2-4% (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was added to freshly foamed gelatine (16.7% (w/v)). Immediately after addition of HA, the mixture was whirled at high velocity to avoid clogging of the gelatine. In order to avoid an inhomogeneous mixture, the temperature should not be below room temperature. After mixing, the mixture was poured into trays or placed on fine perforated teflon sheets, followed by gentle freeze-drying (product temperature: 30°C after 7 hours). Sponges prepared this way had a more porous structure as compared to the sponges prepared in Example 1. Moreover, it was found that this method was suitable for preparing sponges having a higher thickness.

Example 3 - Preparation of sponge #3

HA gel (2-4% (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was smeared on the upper surface of a ge atine sponge (Surgifoam®) followed by heat drying at approximately

30°C and 10% relative humidity for about 16 hours. Sponges prepared this way typically had a HA content of at least 13% (w/w).

Example 4 - Determination of water absorption according to USP 24

Sponge #1 was subjected to the water absorption test (USP 24) described above. The USP 24 method is intended for absorbable gelatine sponges without HA. In the USP 24 method, kneading of the sponges has been optimised with respect to gelatine sponges. For the sponges of the invention, the kneading should preferably be more gentle.

The absorption properties were compared to a commercially available gelatine sponge Surgifoam®. The following results were obtained:

	Sponge #1	Gelatine sponge
	Relative absorption (g/g)	Relative absorption (g/g)
	30.4	60.0
	29.5	57.9
	27.1	50.0
	24.1	54.9
	25.1	59.9
Mean:	27.2	56.5
SD:	2.7	4.0

As can be seen, the ratio between the water absorbed by sponge #1 and the water absorbed by the absorbable gelatine sponge (Surgifoam®) was 27.2/56.5 = 0.48.

In addition, by visual inspection of the wetted sponges, it was evident that sponge #1 swelled to a significantly lesser extent than did the Surgifoam® sponge.

Example 5 - Evaluation of haemostatic efficacy in a porcine spleen model

The object of this study was to compare the efficacy of the sponges of the invention as compared to commercially available gelatine sponges (Surgifoam®) when applied to small, freely bleeding incisions made in the spleen of a pig. The sponges (6.5 cm^3) were applied after pre-wetting in sterile saline, followed by kneading as gentle as possible.

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The pig was the animal of choice since it has a large blood volume and a large vascular spleen that enables many haemostatic comparisons on each animal. The sponges were applied one after one to multiple surgical incisions in the spleen during the test period. The pigs were euthanized and not allowed to recover from anaesthesia.

The primary test parameter was time to haemostasis.

A midline abdominal incision was made to expose the spleen. 1.0 cm incisions (2 mm deep) were made in the spleen. Two incisions were made to demonstrate consistent bleeding with saline-moistened gauze and three incisions were made for each sponge to be tested.

The test sponge (or control) was applied with digital pressure for 2 minutes. Haemostasis evaluation occurred every 30 seconds with an additional 30 seconds of digital pressure. A negative control using saline-moistened gauze was performed at the start and at the end of the study to demonstrate consistent bleeding of >12 minutes in the absence of a haemostatic agent.

A trial was stopped if the bleeding did not stop within at least 5 minutes and/or if the sponge was saturated without reducing bleeding during the previous inspections.

The following results were obtained:

Sponge	Time to haemo-stasis (min)	Surgeon's comments
Negative control	>12	
Gelatine sponge	>6	Trial stopped because of saturated sponge and no reduced bleeding observed. The incision size was 1.5 cm in this trial. From this stage the incision size was reduced to 1.0 cm.
Gelatine sponge	>5	Almost no bleeding. Trail stopped because of saturated sponge and no reduced bleeding was observed.
Gelatine sponge	>5	Trail stopped because of saturated sponge and no reduced bleeding was observed.

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Sponge	Time to haemostasis (min)	Surgeon's comments
Sponge #1	4	Pre-wetting slow compared to Surgifoam®. Good adherence to surface. Only superior absorption in the sponge.
Sponge #1	4	Pre-wetting slow compared to Surgifoam® and becomes less soft by pre-wetting, but appears good enough. Only superior absorption in the sponge.
Sponge #1	3	Only superior absorption in the sponge.
Sponge #1	5	Pre-wetting slow compared to Surgifoam®. Good adherence to surface. Only superior absorption in the sponge.
Sponge #1	3	Pre-wetting slow compared to Surgifoam® and becomes less soft by pre-wetting. Only superior absorption in the sponge.
Sponge #1	2	
Sponge #3*	>5.5	Reduced to moderate bleeding. Trial stopped because of saturated sponge and no reduced bleeding was observed.
Sponge #3*	>5	Trial stopped because of saturated sponge and no reduced bleeding was observed. Easy to pre-wet.
Sponge #3*	>5	Almost no bleeding. Product saturated after 5 minutes.
Sponge #3**	5	Only superior absorption in the sponge.
Sponge #3**	5	Slight absorption within the sponge.
Sponge #3**	3	Only superior absorption in the sponge
Negative control	>12	

*: HA layer away from bleeding

**: HA layer towards bleeding

The reason for Surgifoam® exhibiting a longer time to haemostasis than seen in previous porcine spleen models could be explained by the relative small size of the sponge (6.5 cm^3).

As can be seen from the obtained results, the haemostatic properties of the sponges of the invention (sponge #1 and #3) were clearly superior as compared to Surgifoam®. In particular, sponge #1 was very efficient having a diversity in mean time to haemostasis of at least 2 minutes as compared to Surgifoam®.

CLAIMS

1. A haemostatic sponge comprising a biologically absorbable solid material and at least 10% (w/w) of hyaluronic acid (HA) or a derivative thereof.

2. The haemostatic sponge sponge according to claim 1, wherein said sponge comprises at least 15% (w/w) of HA or a derivative thereof, such as at least 20% (w/w) of HA or a derivative thereof, e.g. at least 25% (w/w) of HA or a derivative thereof, preferably at least 30% (w/w) of HA or a derivative thereof, such as at least 35% (w/w) of HA or a derivative thereof, e.g. at least 40% (w/w) of HA or a derivative thereof.

3. The haemostatic sponge according to claim 2, wherein said HA derivative is a salt or an ester of HA.

4. The haemostatic sponge according to any of the preceding claims, wherein said biologically absorbable solid material is selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof.

5. The haemostatic sponge according to claim 4, wherein said biologically absorbable solid material is gelatine.

6. The haemostatic sponge according to claim 5, wherein said gelatine is denatured.

7. The haemostatic sponge according to any of the preceding claims, wherein said sponge comprises at the most 85% (w/w) of said biologically absorbable solid material, such as at the most 80% (w/w) of said biologically absorbable solid material, e.g. at the most 75% (w/w) of said biologically absorbable solid material, preferably at the most 70% (w/w) of said biologically absorbable solid material, such as at the most 65% (w/w) of said biologically absorbable solid material, e.g. at the most 60% (w/w) of said biologically absorbable solid material.

8. The haemostatic sponge according to any of the preceding claims, wherein said HA, or a derivative thereof, is incorporated in said sponge.

9. The haemostatic sponge according to any of claims 1-7, wherein said HA, or a derivative thereof, is applied to one or more of the surfaces of the sponge.

10. The haemostatic sponge according to any of the preceding claims, which further comprises at least one blood coagulation factor.

11. The haemostatic sponge according to claim 10, wherein said blood coagulation factor is selected from the group consisting of thrombin or a precursor therefor, factor Va, factor VIIa, factor VIIIa, factor IXa, factor Xa, factor XIa, factor XIIa, factor XIIIa and calcium ions.

12. The haemostatic sponge according to claim 11, wherein said blood coagulation factor is thrombin or a precursor therefor.

13. The haemostatic sponge according to claim 12, wherein said blood coagulation factor is thrombin.

14. The haemostatic sponge according to any of claims 10-13, which further comprises a thrombin-stabilising agent selected from the group consisting of naturally occurring amino acids, mono- or disaccharides, polyglycols, proteins and mixtures thereof.

15. The haemostatic sponge according to any of the preceding claims, which further comprises at least one anti-fibrinolytic agent.

16. The haemostatic sponge according to claim 15, wherein said anti-fibrinolytic agent is selected from the group consisting of aprotinin, pepstatin, leupeptin, antipain, chymostatin, gabexate mesilate, fibronectin, ε-amino caproic acid and tranexamic acid.

17. The haemostatic sponge according to claim 16, wherein said anti-fibrinolytic agent is tranexamic acid.

18. The haemostatic sponge according to any of the preceding claims, wherein said sponge absorbs less water than an absorbable gelatine sponge, such as Surgifoam®.

19. The haemostatic sponge according to claim 18, wherein the ratio between the water absorbed by the haemostatic sponge according to any of claims 1-17 and the water absorbed by an absorbable gelatine sponge, such as Surgifoam®, is at the most 0.80 when determined in accordance with USP 24.

20. The haemostatic sponge according to claim 19, wherein said ratio is at the most 0.75, such as at the most 0.70, e.g. at the most 0.65, preferably at the most 0.60, such as at the most 0.55, in particular at the most 0.50.

21. The haemostatic sponge according to any of the preceding claims, wherein at least one of the surfaces of said haemostatic sponge is covered by a top sheet.

22. The haemostatic sponge according to claim 21, wherein said top sheet is removable.

23. The haemostatic sponge according to claim 21 or 22, wherein said top sheet is prepared from a non-biodegradable material.

24. The haemostatic sponge according to claim 21 or 22, wherein said top sheet is prepared from a biodegradable material.

25. Use of a haemostatic sponge according to any of the preceding claims as a haemostatic adjunct in medical, veterinary or dental surgery.

26. Use of a haemostatic sponge according to any of claims 1-24 for the preparation of a haemostatic adjunct to be used in medical, veterinary or dental surgery.

27. A method for arresting bleeding comprising applying to the site of bleeding a haemostatic sponge according to any of claims 1-24.

28. A method for preparing a haemostatic sponge according to claim 8, said method comprising the steps of:

i) providing a whipped or foamed solution of a biologically absorbable solid material selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof;

ii) providing a solution of HA or a derivative thereof;

iii) mixing the solutions provided in i) and ii) above; and

iv) drying said mixture.

29. The method according to claim 28, wherein said biologically absorbable solid material is gelatine.

30. The method according to claim 28 or 29, wherein said solution of HA, or a derivative thereof, is provided in the form of a gel.

31. The method according to any of claims 28-30, wherein said mixing is performed under vigorous mechanical influence, such as whipping, stirring, spinning or centrifugation.

32. The method according to any of claims 28-31, wherein said drying is performed at a temperature from about 25°C to about 35°C, such as at about 30°C.

33. The method according to any of claims 28-32, wherein said drying is conducted for about 12 to about 24 hours, such for about 16 hours.

34. The method according to any of claims 28-31, wherein said mixture is freeze-dried.

35. A method for preparing a haemostatic sponge according to claim 9, said method comprising the steps of:

i) providing a sponge comprising a biologically absorbable solid material selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, polyglycolic acid, polyacetic acid and mixtures thereof;

ii) providing a solution of HA or a derivative thereof;

iii) applying said solution of HA, or a derivative thereof, to one or more of the surfaces of the sponge; and

iv) drying the resulting sponge.

36. The method according to claim 35, wherein said biologically absorbable solid material is gelatine.

37. The method according to claim 35 or 36, wherein said solution of HA, or derivative thereof, is provided in the form of a gel.

38. The method according to any of claims 35-37, wherein said drying is performed at a temperature from about 25°C to about 35°C, such as at about 30°C.

39. The method according to any of claims 35-38, wherein said drying is conducted for about 12 to about 24 hours, such for about 16 hours.

40. The method according to any of claims 35-37, wherein said drying is performed by means of freeze drying.

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